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SUBJECT: GARLON (triclopyr): Review of Mutagenicity Study, Submitted December 21, 1979; Status on Completion of Testing (Meeting of February 19, 1980).

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TO: Edwin R. Budd
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The following mutagenicity study was examined with reference to completion of a Heritable Translocation Test:

"DOWCO 233: Dominant Lethal Evaluation in Mice. Interim Report," by T.R. Hanley, Jr., J.S. Murray, W.C. Hayes, ~~W.C. Hayes~~, J.A. John and K.S. Rao (Dow Chemical, December 13, 1979).

Background: This interim report presents of data from the dominant lethal portion of a study protocol designed to test for one type of inherited chromosomal aberration (Heritable Translocation Test). Based on the results of this dominant lethal portion (reported as negative), Dow requested a meeting to discuss the future of the main study. (Properly conducted, the protocol for the heritable translocation study would have generated about 300 F_1 males per dose for analysis.)

In consideration of the large magnitude in time and resources required to complete the full study, DOW's director for that study, Dr. K.S. Rao, and their registration specialist, Mr. Wayne Stringer, met with Mr. Richard Mountfort and myself on February 19, 1980. The following were discussed at that meeting:

(1) My preliminary evaluation of the dominant lethal portion of the study;

(2) Status on completion of the translocation test;

(3) Results of previous mutagenicity testing of this product, including an earlier rat study which revealed weak evidence of dominant lethality.

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The conclusions of this meeting were:-

(1) Satisfactory resolution of most of the questions raised in my preliminary evaluation of the dominant lethal portion, indicating a valid study had been performed, and a valid finding had been generated. Dow, however, was requested to supply additional data (see: Recommendations).

(2) Based on the negative findings of the mouse dominant lethal under review, completion of the heritable translocation assay would not add useful information on the genetic end-point being assayed in this species (gross chromosomal aberrations, indication for which would have surfaced in the dominant lethal portion were they induced). Due consideration was given to reviews of the protocol by outside expert consultants before the study was begun, as well as by Dr. Sidney Green, Chief to the Toxic Effects Branch, HRD, who has conducted both types of assays.

(3) The results of other mutagenicity studies submitted by DOW were reviewed, and accepted as support for their conclusion that triclopyr is probably not mutagenic in the tests conducted. These studies were performed for DOW by Litton Bionetics in 1973, with reported negative findings in three tests: (i) an Ames assay by the disc (spot) method; (ii) in vivo cytogenetics in rats, using both acute and subacute dosage schedules orally; (iii) subacute host-mediated assay with mice dosed orally. An additional Ames assay has been requested, however (see below: Recommendations).

Evaluation of the Study: "DOWCO 233: Dominant Lethal Evaluation in Mice. Interim Report."

Protocol: DOWCO 233 (triclopyr, or 3,5,6-trichloro-2-pyridyloxyacetic acid = Garlon 3A Herbicide) was administered to groups of 30 adult male CF-1 mice in the diet for a period of nine weeks at dose levels of 0, 3, 15 or 70 mg/kg/day. Dietary concentrations were adjusted on a weekly basis using weekly food consumption and body weight gains for each dose group. An additional group of 30 mice was used as a positive control, receiving a single intraperitoneal injection of 0.30 mg/kg triethylenemelamine (TEM), a known mutagen for mice, 24 hours prior to termination of the treatment period.

Each male was then housed individually with four adult virgin untreated females for one week, followed by a second week of matings with different females. At the completion of each breeding period, two of the four females were selected at random for assignment to the translocation study; the remaining pair was assigned to the dominant lethal study. Ten days after the last day of cohabitation, females

assigned to the dominant lethal portion were sacrificed, and resorptions recorded for each female. Uteri of apparently non-pregnant females were stained with sodium sulfide (to visualize implantation sites) and the numbers of resorptions recorded and included in the statistical evaluations.

Statistical Procedures: Weekly body weights, food consumption and the number of implantations were evaluated by one-way analysis of variance; differences from control were examined using Dunnett's Test. The fertility index was analyzed by the Fisher Exact Probability Test, and the resorption rate by the Wilcoxon (non-parametric) Test. The level of significance chosen for all cases was $p<0.05$; statistical outliers ($p<0.02$) were excluded for food consumption by the appropriate Grubbs method. Data were evaluated using males (fertility index, Table I of report), and/or females (implantations and resorptions, Tables II and III of report) as experimental units.

Results: Tables I through III report no statistical differences for any reproductive parameter at any of the dose levels of DOWCO 233 employed when compared to controls. In contrast, statistically significant differences from control values were reported for the TEM positive control group, as follows: (i) decrease in implantations for the second week of breeding; (ii) increases in resorption rates for both breeding periods.

The conclusion drawn from these data was that DOWCO 233 did not induce dominant lethals in CF-1 mice when administered in the diet at dose levels of 3, 15, or 70 mg/kg/day over a period of nine weeks.

Reviewer's Comment: This study appears to have been performed in a manner consistent with the generation of valid results. The statistical tests selected are appropriate for the individual parameters measured. The experimental design for dosing, i.e., in the diet continuously for 9 weeks (one complete spermatogenic cycle), is a modification of the standard protocol for this type of test (acute or 5-day subacute dosage), but has been shown to produce positive results with known mutagens (e.g., TEM) in both rats and mice, with no loss of pertinent information except that of germinal stage sensitivity. Sampling is necessarily restricted to only the first few weeks following termination of treatment with this modification, but apparently this has been sufficient to detect most mutagens.

To account for the discrepancy between the negative result in mice (this report) and the weakly positive dominant lethal effect previously reported in rats, the registrant offers the totality of negative mutagenicity testing results previously submitted (Ames "spot" test; in vivo cytogenetics; host-mediated assay), and suggests the weak response in rats was due to chance. It is possible for the dominant lethal assay to generate statistically significant random positive results in some sampling weeks, due to the large variances calculated for the

reproductive parameters when small numbers of animals are used. Only 10 males per group were treated in the rat study, compared to 30 per group in this mouse study. Equivalent dosages were used in both studies (ranging up to 70.0 mg/kg/day), although DOWCO 233 appears to be more toxic for mice (acute oral LD_{50} = 471 mg/kg, compared to 713 mg/kg for rats). Although statistically significant positive results were calculated for some reproductive parameters (average number of resorptions, proportion of females with 2 or more resorptions) for both higher doses of DOWCO as well as the positive control (TEM), there was inconsistency with respect to both sampling weeks with such positive effects in other parameters (e.g., ratio of dead implants); in the latter case, for example, TEM was judged not positive for breeding week 5, where DOWCO-233 registered positive.

Recommendations:

(1) Since the dominant lethal portion of the protocol for conducting a heritable translocation test in mice gave negative results, i.e., no indication of chromosome aberrations leading to embryonic lethality, completion of the full study designed to assay for only those aberrations leading to sterility would not reasonably be anticipated to provide useful information. Administration of the test substance continuously over the entire spermatogenic cycle of the mouse at the dosages employed was a valid test for dominant lethal mutations.

(2) To complete the record for this study, however, DOW was requested to submit historical data pertaining to reproductive parameters on the CF-1 mouse strain, as well as surviving reproductive data (litter size, etc.) for the balance of the animals assigned to the translocation test.

(3) In addition, DOW was requested to provide a new Ames assay using the plate incorporation method. A negative result by the disc method ("spot") is not sufficient to characterize the test material as non-mutagenic in this type of test.

cc: Robert J. Taylor (RD/TS-767)
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